Experimental Biology and
Medicine Institute

May 12, 1949

Dr. Fritz Lipmann
Biochemical Research Laboratory
Massachusetts General Hospital
Boston 14, Massachusetts

Dear Dr. Lipmann:

I hope you will forgive my delay in sending you the enzyme and then not describing the preparation. Since returning from Detroit, I have been working rather intensively on its preparation and during the last few days on some studies of its activity.

The preparation that was sent you contained 500 units per cc and its "purity" is 2000 units per milligram protein. A unit has been defined as one micromole DPN split per hour at 38° at pH 7.4. In view of the fact that practically all my experience with this enzyme has been with its DPN-splitting properties, I would consider that the most safe criterion of its potency. However, the rate of increase of fluorescence of a FAD preparation or the rate of hydrolysis ATP may turn out to be equally reliable. The preparation you have contains approximately 125 units per cc with respect to micromoles of phosphate release from ATP. Thus, the relative potency of DPN to ATP splitting is approximately 4. The ratio of 300, as indicated in my note, is misleading in that the ATP units were differently defined; they were expressed in terms of a reaction velocity constant.

With regard to monoesterase activity of the preparation, it is roughly one per cent of the potency indicated for the following substrates: Yeast adenylic acid, muscle adenylic acid, nicotinamide mononucleotide, inorganic pyrophosphate, glucose-6-phosphate, and glycerophosphate. As I mentioned to you in Detroit, the affinity of DPN for the enzyme is rather high and is a strong competitive inhibitor in the splitting of FAD, ATP and TPN, but does not depress the rate of splitting of inorganic pyrophosphate or the monoesters mentioned.

A word about the stability of the enzyme. Solutions containing one milligram protein per cc or more have been perfectly stable for over a year at 0 to 5°. It has been rather astonishing to test some preparations of April 1948 in which dark precipitates had appeared and find that their activity when retested was identical to their original activity. However, some highly diluted samples (less than 40 gamma per cc) have lost half their potency in a week.

Since sending you the small sample of material, we have completed a rather large preparation and could spare a great deal of material of approximately the same purity. Please do not hesitate to write me when your supply is exhausted. I hope that I shall have some more data on this enzyme within a couple of weeks, and I will send you a copy of the manuscript at the earliest possible date.

With fond regards,

Sincerely yours,

Arthur Kornberg.

AK:T

Air Mail